USE OF ANTIFOAM IN AN AIRLIFT BIOREACTOR FOR THE PRODUCTION OF BIOHERBICIDE

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ABSTRACT

In the development of bioprocesses that involve scaling up fermentations, choosing a bioreactor and studying parameters (such as temperature, agitation, aeration, among others) are fundamental to ensure the product quality. This study evaluates the use of antifoam and its effect on parameters of the fermentation process of a fungal extract using microalgal biomass as a substrate in an a Airlift bioreactor, aiming to produce bioherbicide. For that, we quantified the production of enzymes involved in the bioherbicide activity (amylase, cellulase, laccase, lipase, and peroxidase). We identified a fermentation condition (412 mL of antifoam; 28ºC; pH of 8.3; 8 LPM of aeration) in which antifoam allowed the production of an enzyme cocktail and did not negatively affect fungal development and enzyme production.

Keywords: Bioprocesses. Fermentative strategies. Process optimization. Bioinputs. Scale-up.

1 INTRODUCTION

The use of bioherbicides can bring a series of benefits to agriculture. This technology effectively reduces food production costs and crop yields and can be added to integrated strategies for managing weeds. The quality of a bioherbicide directly depends on how the fermentation process is conducted, in which the microorganism needs specific nutrients and appropriate conditions for its development[1.]

One of the current challenges for popularizing bioherbicides is to make their production method economically viable. To do so, it is necessary to understand how parameters (i.e., temperature, agitation, pH, oxygen availability) affect the production of secondary metabolites, which are responsible for the phytotoxic effects caused in target plants[2]. The fermentation conditions can be adjust according to expectations, directing the composition of the final product[3].

Foam formation may occur during fermentation, resulting from protein-protein interaction or interaction with other molecules present in the medium. Sometimes the foam demonstrates stability and persistence, in these cases, the use of antifoam is essential to preserve the bioprocess and the equipment[4,5]. Furthermore, in airlift bioreactors, the agitation of the medium occurs with the upward entry of air, which can favor the formation of foam in the fermentation medium, if it already has characteristics that predispose the occurrence of foam, such as interaction with proteins[6].

Therefore, this study aims to study the use of antifoam and its effect in the fermentatios of a fungal extract using microalgal biomass as a substrate in an Airlift bioreactor. In response, we quantified the production of enzymes involved in bioherbicidal activity (amylase, cellulase, laccase, lipase, and peroxidase)[7,8] in extracts obtained by different fermentations.

2 MATERIAL & METHODS

The microalgae used as fermentative substrate belong to the genus *Chlorella* spp. and come from the phytoremediation of wastewater from biogas production (digestate), implemented at EMBRAPA Swine and Poultry (Concordia, SC, Brazil)[9]. The fungus used in this study was *Trichoderma koningiopsis* (identification code in GenBank MK860714).

The fermentations occurred in an benchtop airlift bioreactor, model Bio-Tec-Pro-II (Tecnal, Brazil), with a working volume of 3.0L. Five different fermentations were done, with a 2.0 L of medium, in which adjustments in the operational parameters were made until an adequate condition for the development of the bioherbicide potential was obtained. The medium comprised 200 g of wet microalgal biomass, and the remainder of the liquid portion was divided between distilled water, inoculum, and antifoam (see Table 1).

As a control, F1 was performed without inoculum, only with the substrate. In F2, an antifoam called Fermax, provided by the Centro de Tecnologia Canavieira (CTC) was used. In testes F3, F4, and F5 was used the antifoam AFP 320 (FAXON Química). Table 1 also shows the total amounts of antifoam used in the fermentations, and the values are arranged as follows: V_i+V_f=total. F3, F4, and F5 were performed with antifoam diluted in the fermentation medium, represented by the initial volume values (V_i). In addition, the antifoam was added during the process (V_f). In F4 and F5, at each sample withdrawal, the same volume of microalgal biomass diluted in distilled water (10% concentration) was added to the bioreactor to evaluate how the process would behave with the reintroduction of the substrate, in fed-batch mode. So that, a volume of fresh substrate equal to the volume of the withdrawn sample.

Table 1 Operating conditions used in the benchtop airlift bioreactor to optimize the fermentation process to obtain bioherbicide potential.

Fermentation	Airflow (LPM*)	Temperature (ºC)	pH**	Antifoam (mL) (Vi+Vf=total)***
F1	2	28	7.0	-
F2	2	28	8.0	0+50 = 50
F3	5	28	7.9	20+131 = 151
F4	5	28	7.8	200+34 = 234
F5	8	28	8.3	400+12 = 412

*Liters per minute
**pH was not controlled. The values refer to the end fermentation process, in 168 h.
*** Antifoam diluted in the fermentation medium, represented by the initial volume values (V_i). Antifoam add during the process (V_f).

The bioreactor was autoclaved at 120°C for 30 min. After sterilization, they were inoculated with 10^6 spores/mL of *Trichoderma koningiopsis* suspension. During fermentation, samples (approximately 20 mL) were taken every 24 h for enzymatic verification (amylase, cellulose, laccase, lipase, and peroxidase)[10-16]. One unit of enzymatic activity (U) corresponds to the formation of one micromol of product per minute by the enzyme, under test conditions.

3 RESULTS & DISCUSSION

Between 72 and 120 h of fermentation, was observed an intensification of foam formation in the medium. This may be linked to the more excellent metabolic activity of the fungus *Trichoderma koningiopsis*, a period in which the fungus assimilates nutrients from the medium[6].

The foam demonstrated stability and persistence, which may indicate the presence of saponins, possibly originating from microalgae. The main characteristic of the existence of saponins is the formation of foam when in contact with water and agitation, which was the situation observed when fermentations occurred in the Airlift bioreactor. Furthermore, as saponins are considered secondary metabolites, they can produce various agro-industrial products, including biopesticides[4]. Foam can also originate from protein-protein interaction, and interaction with enzymes produced during fermentation[5]. Antifoam did not negatively affect fungal development and enzyme production; however, it enabled fermentations to occur in this bioreactor.

Fermentations continued for 168 hours until the enzymatic activities in the culture broth reached constant values. The highest enzymatic activities occurred between 120 and 168 h, may be related the fungus's more excellent metabolic activity, followed by enzymatic activities and foam formation. Fermentations F4 and F5 gave the highest enzyme activities, which can be linked to the reintroduction of substrate during the fermentation.

During F5, dissolved oxygen remained close to 100% throughout the process. The aeration of 8 LPM, the reintroduction of a substrate, and the volume of defoamer enabled an optimized system, which benefited an excellent adaptation of the microorganism to the process, also providing a favorable environment for the production of enzymes (Figure 1).

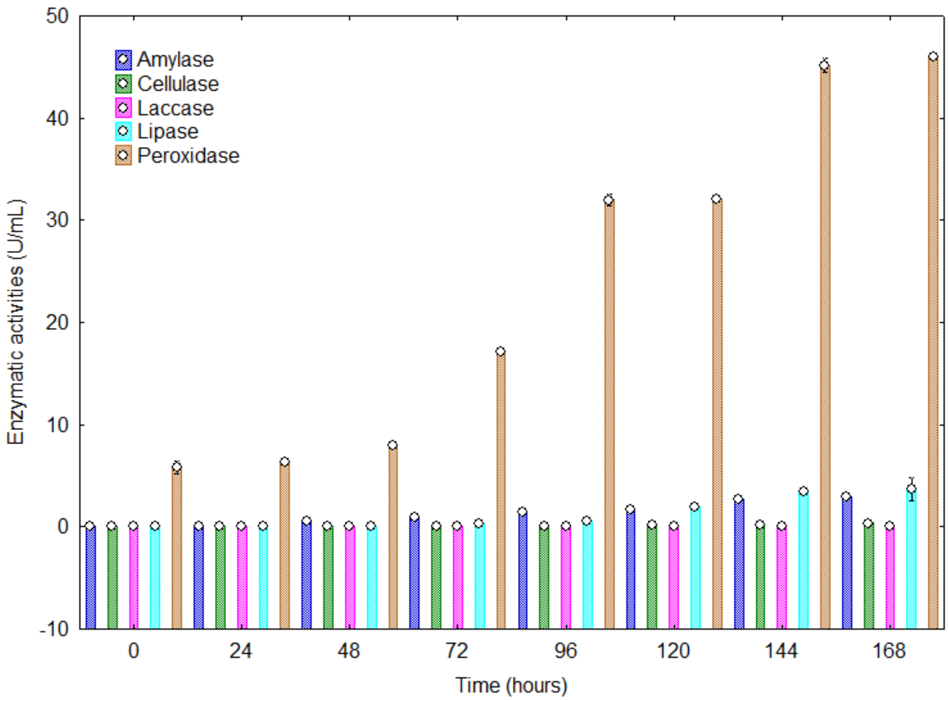

Figure 1 Results of production of amylase, cellulase, laccase, lipase, and peroxidase, in fermentation F5 (optimized condition), carried out in the airlift bioreactor, during 168 h of fermentation.

The pH of the medium increased with the addition of antifoam, which may be due to the accumulation of protein particles and changes in surface hydrophobicity[17]. The pH values of antifoam vary between 6.5 and 8.3, which corroborates the medium's pH values, which increase with the addition of antifoam[18]. Furthermore, other studies that investigated the enzymatic production of *Trichoderma koningiopsis* using microalgae biomass as substrate found that at pH 8.5, it was possible to obtain an enzymatic pool (amylase, cellulase, lipase, laccase, and peroxidase) and an ideal value of fungal biomass for application as a potential bioherbicides[8].

4 CONCLUSION

The results obtained in this study expand our knowledge about antifoam's beneficial effects in bioherbicide production using airlift bioreactors. Also, we can associate the production of enzymes with fermentative conditions such as aeration, dissolved oxygen, antifoam, pH, microorganism, and substrate.

The results suggest that use of antifoam allowed fermentation to occur, taking into account its characteristics of fermentative medium, microorganism and type of bioreactor, presenting the possibility of large-scale production of a potential bioinput, providing scientists in this field with innovative and more sustainable management tools.

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